Yeast Expression Testing Protocol

1) Grow beast on SD-CAA (glucose)
    a) 3 mL of SD-CAA (drop out media) + 30 microliters yeast for 24 hrs
2) Create glucose and galactose media
    a) Filter sterilize a 20% stock of sugar solution (glucose or galactose, that is 20 g sugar/ 100 mL)
    b) Make synthetic defined (SD) base (per 900 mL):
        i) 14.7 g sodium citrate
        ii) 6.7 g yeast N2 base without amino acids (https://www.sigmaaldrich.com/catalog/product/sigma/y0626?lang=en&region=US this or similar)
        iii) 3.82g dropout powder – this has all amino acids and maybe uracil EXCEPT for what you're using as an auxotrophic marker on your vector, i.e. the amino acid that is dropped out. I've personally used optimized custom blends (SD-2xSCAA – see paper), but given the time, one of the synthetic drop-out medium supplements on this page will likely do -https://www.sigmaaldrich.com/technical-documents/articles/biology/Introduction-yeast-media.html
        iv) 4.2g citric acid monohydrate
        v) Filter sterilize (You can potentially pH buffer but it's not in my old recipe and I don't recall doing it….)
    c) Aseptically mix 900 ml of SD base with 100 ml of sugar (i.e. dilute sugar 10-fold with SD base)
    d) Use for growth
3) Measure OD
4) Pellet, decant, wash with fresh PBS, resuspend (pipette), pellet, decant, resuspend
5) Put 50 milliliters of glucose and galactose media into separate "erlenmeyer flasks"
6) Put OD .1 worth of cells into both "erlenmeyer flasks"
7) Remove after 4 hours, 8 hours and 24 hours
8) Each time removed measure the OD (centrifuge 10ng for 1 min)
9) Freeze (-80˚C) sample volume so that sample volume X OD = 5
10) Sample from galactose and glucose weill have different OD's
11) Don't use glycerol